BANANA GUARD

Protocols

Transformations

Chemical Competent Cells Buffers

Buffer 1:

- 1 M $MgSO_4$
- 1 M $MgCl_2$

Buffer2:

- 100 mM RbCl
- 50 mM $MnCl_2$
- 10 mM $CaCl_2$
- 15% glycerol
- pH=5.8

Buffer3:

- 10 mM MOPS
- 10 mM RbCl
- 75 mM $CaCl_2$
- 15% glycerol
- pH=6.8

Chemical Competent Cells Production Protocol

Making chemical competent cells protocol
Inoculate 40 ml LB with 1% o/n E.coli culture + 1%
Buffer 1
Grow the culture at 37°C aerated until an OD_{600} of 0.4-0.5 is reached.
Incubate the culture on ice 15'at 5°C
Centrifuge the culture 15' , 3600 rpm, 4°C
Resuspend the pellet at 1/3 Vol. (13.33 ml)
Buffer 2(pre-chilled at 5°C)
Incubate the cells 60' at 5°C
Centrifuge the cells 10', 3600 rpm, 4°C
Resuspend the pellet at 1/10 Vol. (4 ml)
Buffer 3 (pre-chilled at 5°C)
Incubate the cells 20' at 5°C
Aliquote the competent cells at 100 ul and immediately snap freeze the cells on dry ice
After 15' store at -80°C

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Transforming chemical competent cells

Thaw the competent cells (100 ul) 10' on ice.
Add DNA (ligation 2 ul) and mix by swirling gently with pipette tip .
Incubate cells 30' @ 5°C (binding of DNA to membrane).
Heat shock cells 45" @ 42°C (not shorter, not longer).
Immediately place cells back on ice, and incubate 5' (cells recover).
Add SOC medium 250 ul (pre-warm SOC at Room Temp).
Incubate cells 45'-60' @ 37°C (not longer, resistance synthesis).

Plate cells on LB-agar with appropriate antibiotic and grow o/n @ 37°C.
e.g. plate 10 ul, 50 ul, 100 and 140 ul cells

Electro competent cells production protocol

Grow cells O/N at 37°C.
Cool down in ice for 5 min
Centrifuge (4700rpm, 5 min)
Discard supernatant and respuspend in 10 mL MQ water
Repeat washing steps 3 and 4.
Centrifuge (4700rpm, 5 min)
Resuspend in 10 mL 10% glycerol
Centrifuge (4700rpm, 5 min)
Resuspend in 100 µL 20% glycerol

Transforming electro competent cells

Add 1 µL of plasmid for transformation
Rest for 10 minutes.
Transfer into a cold electroporation cuvette (stored in the fridge).
Electroporate at 25 µF, 25 kV and 200 Ω.
Add 900 µL of SOC medium preheated at 37°C.
Incubate at 37°C
Plate 100 µL on a agar plate containing the desired antibiotics.

Using DNA from iGEM plates

(Adapted from iGEM website)

To use the DNA in the Distribution Kit, follow these instructions:

- With a pipette tip, punch a hole through the foil cover into the corresponding well of the part that you want. Do not remove the foil cover, as it could lead to cross contamination between the wells.
- Pipette 10 µL of distilled water into the well. Pipette up and down a few times and let sit for 5 minutes to make sure the dried DNA is fully resuspended.
- Transform 1 µl of the resuspended DNA into your desired competent cells, plate your transformation with the appropriate antibiotic and grow overnight.
- Pick a single colony and inoculate broth with the correct antibiotic and grow O/N.
- Use the resulting culture to miniprep the DNA and make your own glycerol stock.